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Original Paper

The Physiological effects of *Piriformospora indica* on some crop plants

b- Alleviation effects on stress by Salinity.

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Abstract

The root endophytic fungus *Piriformospora indica* has been shown to increase resistance against abiotic salinity stress in some crop plants: *Vicia faba*, *Lupinus termis*, *Arachis hypogaea* and *Hibiscus sabdariffa*. The presence of the fungus reduced the effect of the salinity on the growth rate; and enhanced the photosynthetic processes and protein contents. Moreover, water relations were improved under the effect of the fungus. It was found that, the treated plants by *Piriformospora indica* reduced the negative effects of salinity on the growth of these plants. From the obtained results, it can be concluded that, *Vicia faba*, *Lupinus termis*, *Arachis hypogaea* and *Hibiscus sabdariffa* can be cultivated in moderated salinity lands in the presence of root endophyte fungus *Piriformospora indica*.

Key words: *Piriformospora indica*, salinity stress, *Vicia faba*, *Lupinus termis*, *Arachis hypogaea* and *Hibiscus sabdariffa*.

Introduction

High salt concentrations in soil and irrigation water are a major threat to agricultural production in arid and semiarid regions. The presence of excess ions in the rhizosphere causes injury to plant roots, followed by their gradual accumulation in the aerial parts with heavy damage to plant metabolism, which leads to stunted growth and reduced yield (Shannon, 1997). Salt stress has been found to disrupt several morphological, physiological and biochemical processes, many of which are seen at plant cellular levels (Maslenkova *et al*., 1999). Many of important salinity effects in plants are including: Hyperionic and hyperosmotic effects, inhibition of enzyme activities in metabolic pathways, decreased carbon-use efficiency, decreased germination percentage, reduction in photosynthesis and the decomposition of protein and membrane structures (Ali Asghar Bagheri *et al*., 2013). Plants have evolved complex mechanisms to counter NaCl toxicity and low water potential in soil caused by salinity as well as drought (reviewed by Munns & Tester, 2008). Furthermore, mutualistic symbiosis with mycorrhizal and endophytic fungi can confer salt tolerance to plants and decrease yield losses in cultivated crops grown in saline soils (Rodriguez *et al*., 2004). Recently, a root-endophytic basidiomycete, *Piriformospora indica*, was used to improve plant resistance against root and leaf diseases and alleviate salt stress in barley (Waller *et al*., 2005).

Piriformospora indica was isolated from the rhizosphere of *Prosopis juliflora* and *Zizyphus nummularia* in the Thar Desert in Rajasthan, India (Verma *et al*., 1998). This fungus colonizes root sand increases the biomass of both monocot and eudicot plants (Varma *et al*., 1999). In contrast to arbuscular mycorrhizal fungi, *P. indica* can be easily grown on synthetic media allowing for large-scale propagation and a possible use in plant production.

Earlier studies were demonstrated that salt-treated barley showed reduction in metabolic activity and respiration rates (Criddle *et al*., 1989; Jolivet *et al*., 1990).

Drought, salt and temperature extremes all induce the accumulation of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals (Apel & Hirt, 2004). Plants are endowed with an array of radical scavengers and antioxidant enzymes that act in concert to alleviate oxidative stress. An imbalance between antioxidant defences and the amount of ROS results in

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cellular injury (Foyer & Noctor, 2000). An increasing body of evidence suggested that high salinity induced oxidative stress in plants that was at least partly responsible for tissue damage (Hernández *et al*., 2000; Mittova *et al*., 2004). Several studies had demonstrated that salinity increases antioxidant activities in salt-tolerant plants above the levels found in salt-sensitive plants (Gossett *et al*., 1994; Gueta-Dahan *et al*., 1997; Mittova *et al*., 2004).

The aim of this study was to investigate the fungus' potential to protect *Vicia faba*, *Lupinus termis, Arachis hypogaea* and *Hibiscus sabdariffa* from salt stress. So plant growth parameters, photosynthetic processes, water relation, protein content and profile, proline content, antioxidant enzyme activity and some element content in plant tissues were determined.

Material and Methods

Plant material:

Seeds of four plants were obtained from the agriculture research center, Shandaweel Agriculture Research Station, Sohag, Egypt. Plants seeds were surface sterilized for 1 min in 75% ethanol and then washed three times by sterilized distilled water for 5 min each.

Soil:

The soil used in the experiments was mixture of sand/clay (2:1) which was sterilized at 180ºC for 30 min in oven before seed sowing.

Cultivation of fungus:

The fungus was maintained on Kafer's medium (Kafer, 1977). The fungus grows in liquid medium. The culture medium was inoculated with agar containing fungal discs and incubated at 28 ± 2ºC under constant shaking conditions (100 rpm) in dark for 14 days.

Experimental design:

The plastic pots containing 2 kg soil were divided into ten groups and treated as the follow (3 pots were used for each treatment):

a- Plants of the 1st group were left without any treatments (control).
b- Plants of the 2nd group were treated with 100 mM NaCl.
c- Plants of the 3rd group were treated with 100 mM NaCl and inoculated with *P. indica.*

Experiment was carried out in the open field greenhouse of Botany department, Faculty of science, Sohag University. Plants were carefully watered every three days with tap water. The previous design was carried out for all plant species.

After 14 days old for plant growth, irrigated plant groups numbered 3 with 200 ml of *P. indica* liquid culture. After other two weeks, irrigated plants groups numbered 2 and 3 by 100 mM NaCl. Then collected the plants after 14 days and did the following experiment:

Growth parameters:

Four weeks after inoculation, whole plants (all species) were harvested and divided into roots and shoots, and fresh & dry weights and length of each were determined.

Determination of photosynthetic process:

a- Photosynthetic pigments:

The photosynthetic pigments viz, chlorophyll a, chlorophyll b and carotenoids, were determined using the spectrophotometric method recommended by Metzner *et al*., (1965). It was possible to determine the concentrations of the pigment fractions (chlorophyll a, chlorophyll b and carotenoids) as mg/ml using the following equations:

Chlorophyll a = 10.3 E_{663} – 0.918 E_{644} = µg/ml

Chlorophyll b = 19.7 E_{644} – 3.87 E_{663} = µg/ml

Carotenoids = 4.2 E452.5– (0.0264 chlorophyll a + 0.0462 chlorophyll b) = µg/ml

Finally, the pigment fractions were calculated as mg/gm fresh weight.

b- Photosynthetic rate and intercellular CO_2:

Leaves of control and treated plants were subjected to analyses of net photosynthetic rate (A) and sub-stomatal CO_2 (Ci) using LCi Portable Photosynthesis System.

Plant-water relationship parameters

a- Transpiration rate and stomatal conductance:

Leaves of control and treated plants were subjected to analyses of net transpiration rate (E) and stomatal conductance (Gs) using LCi Portable Photosynthesis System.

b- Relative water content (RWC):

The RWC stated by Slatyer in 1967, express the in percentage the water content at a given time and tissue as related to the water content at full turgor:

RWC = (FW-DW)/ (TW-DW)

FW = fresh weight
TW = turgid weight
DW = dry weight

Protein content and profile:

a- Protein content:

Total soluble, insoluble and total proteins were determined using the method of Lowry *et al*. (1951). Dry plant material (0.5 g) was homogenized in 5 ml phosphate buffer (pH = 7.0) and centrifuged. The extractants were treated with appropriate reagents and the optical densities were read at 570 nm.

b- Protein profile:

Electrophoresis detection of protein in plant tissue by sodium dodycle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) following the method described by Laemmli (1970) was used in the present study.

Free proline

Proline was determined following Bates *et al*. (1973). Dry plant material (0.5 g) was homogenized in 10 ml of 3% sulfosalicylic acid and the homogenate filtered. The filtrate (2 ml) was treated with 2 ml acid ninhydrin and 2 ml of glacial acetic acid, then with 4 ml of toluene. Absorbance of the colored solutions was read at 520 nm.

Antioxidant enzyme activity:

a- CAT assay.

CAT activity was assayed by measuring the initial rate of H_2O_2 disappearance using the method of Beers & Sizer (1952). 1 ml of catalase assay reaction mixture contained 0.05 mM sodium phosphate buffer (pH 7.0), 20 ml enzyme extract and 1 mM H_2O_2. The decrease in H_2O_2 was followed by a decline in A240, and the activity [U (mg protein)$^{-1}$] was calculated using a molar absorption coefficient of 40 mM^{-1} cm^{-1} for H_2O_2.

b- Peroxidase assay.

The peroxidase activity was determined as described by Machly and Chance (1954). A 0.5 gm of fresh leaf sample was weighed and ground well in a mortar with little quantity of chilled phosphate buffer at pH 6.6 and filtered through a double layered muslin cloth to remove the pulp, made up to 25 ml and centrifuged for 30 minutes at 2000 rpm at 4 ºC. The clear extract was used as enzyme source. 3 ml of 0.05 M guaiacol solution was pipetted out into a test tube to which 0.1 ml of enzyme extract was added. Then 0.5 ml of 1 per cent hydrogen peroxide was added, mixed the contents rapidly and the absorbance was measured in calorimeter at 470 nm at an interval of 20 seconds.

Enzyme activity was calculated by taking the average difference of O.D between two consecutive time intervals and enzyme activity was expressed in terms of OD sec^{-1}mg^{-1} protein (i.e. specific activity).

Mineral elements in plant tissues

The dried and ground samples of leaves (0.3 g each) were digested with sulphuric acid and hydrogen peroxide according to the method of Wolf (1982). Na, K, and Ca were determined with a flame photometer (Jenway PFP-7).

Statistical analysis

Data for all attributes were subjected to a ANOVA one-way analysis of variance and the mean values were compared with the least significance difference (LSD) at 0.05 and 0.01 levels, with the Origin program.

Results

Growth:

To study the ability of colonized plants by *P. indica* to tolerate salinity stress, we were designed experiment for this aim. Plants divided to three groups: 1st were controls, 2nd plants were treated with 100 mM and 3rd plants were treated with *P.indica* after 2 weeks treated by 100 mM. At the end of experimental period growth parameters were measured for both roots and shoots. In generalist, salinity treated plants show significant decreases in fresh, dry weight and length for both roots and shoots compared to control plants. In contrast, treated plants by *P. indica* and salinity appear significant increase in fresh, dry weight and length for both roots and shoots compared to control plants and plants which treated by saline solution.

Moreover, Root fresh weight decreasing ratios in group two (treated by 100 mM NaCl) compared to those in group one (control) were: 14%, 9%, 19% and 32%, and root dry weight were 16%, 24%, 18% and 6%, and shoot fresh weight were 19%, 26%, 2% and 26%, and shoot dry weight were 18%, 35%, 7% and 2% , in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively. For root and shoot lengths there is no general trend on effect of salinity on these parameters, but depend on plant identity. In opposite side, plants belong to group three (*P. indica* + 100 mM NaCl) show enhancement in growth parameters

compared to plants in group two (100 mM NaCl), where root fresh weight increasing ratios were 62%, 149%, 80% and 87%, and root dry weight were 19%, 107%, 47% and 73%, and shoot fresh weight were 86%, 39%, 103% and 92%, and shoot dry weight were 44%, 61%, 109% and 30%, root length were 40%, 22%, 7% and 12%, and shoot length were 25%, 10%, 37% and 2%, in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively (Fig. 1).

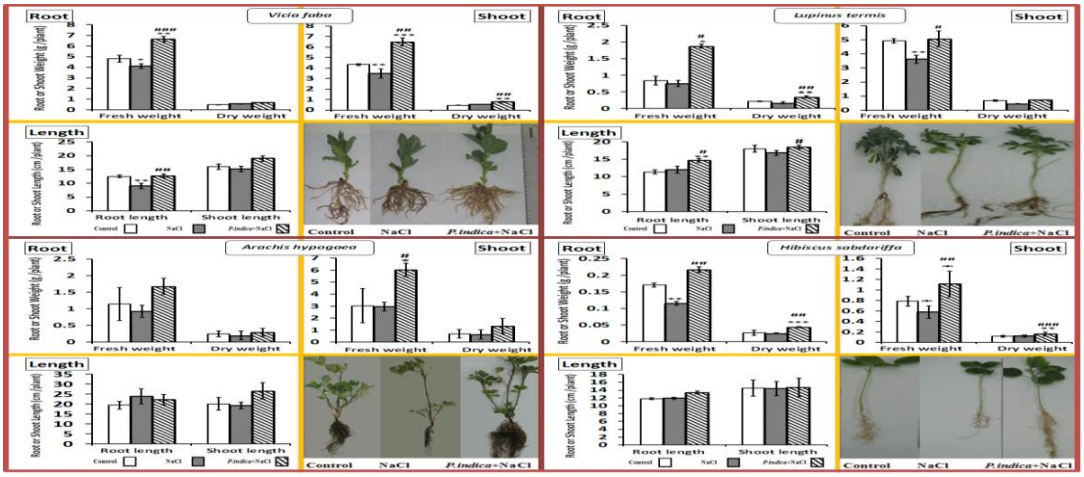

Figure 1: Effect of salinity stress (100 mM NaCl), and inoculation with *P. indica* under salinity stress (100 mM NaCl) on root and shoot fresh, dry weight and length of experimental plants. Error bar in each column equals the standard deviation from the mean. Difference is significant at P<0.05 (*), at P<0.01(**), and at P<0.001 (***) (compared with control plants). Difference is significant at P<0.05 ($^{\#}$), at P<0.01 ($^{\#\#}$), and at P<0.001 ($^{\#\#\#}$) (Compared with treated plants with 100 mM NaCl), according the One-Way ANOVA.

Photosynthesis:

Recorded results for photosynthetic pigments content in fresh leaves plants tissue represented by chlorophyll a, chlorophyll b and carotenoids appeared significant decrease in group two plants (treated by 100 mM NaCl) compared to control plants. Moreover, plants in group three (colonized by *P. indica* + treated by 100 mM NaCl) recorded enhancement in photosynthetic pigments content compared to group two plants (treated by 100 mM NaCl) in all plant species. Moreover, decreasing ratio in total pigments content in group two plants (treated by 100 mM NaCl) compared to control plants were; 5%, 14%, 3% and 26%, in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively. Furthermore, enhancement in total pigments contents in group three plants (colonized by *P. indica* + treated by 100 mM NaCl) compared to group two plants (treated by 100 mM NaCl) were; 7%, 54%, 11% and 32%, in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively (Fig. 2).

Photosynthetic rate:

Under effect of salinity, photosynthetic rate appear negative effect in group two plants (treated by 100 mM NaCl) compared to control plants were; 35%, 35%, 34% and 18%, in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively. Moreover, colonized plants can overcome on bad effect of salinity by enhancing the photosynthetic rate. where, significant increasing in group three plants (colonized by *P. indica* + treated by 100 mM NaCl) compared to group two plants (treated by 100 mM NaCl) reach up to 35%, 137%, 57% and 93%, in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively (Fig. 2).

Intercellular CO_2:

Furthermore, the intercellular CO_2 concentration (Ci) showed non-significant change in cases group two compared to group one plants and group three compared to group two plants (Fig. 2).

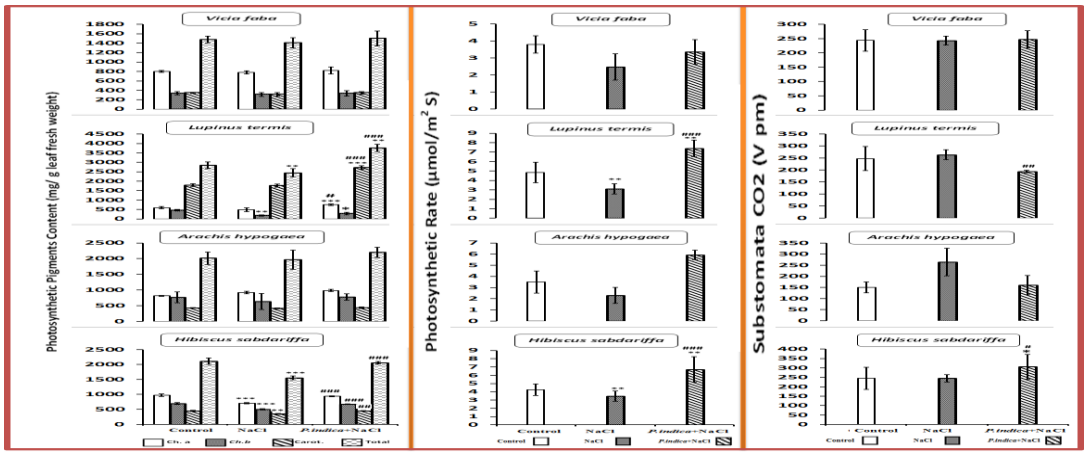

Figure 2: Effect of salinity stress (100 mM NaCl), and inoculation with *P. indica* under salinity stress (100 mM NaCl) on leaf content of photosynthetic pigments, photosynthetic rate and intercellular CO_2 of experimental plants. Error bar in each column equals the standard deviation from the mean. Difference is significant at P<0.05 (*), at P<0.01(**), and at P<0.001 (***) (compared with control plants). Difference is significant at P<0.05 ($^{\#}$), at P<0.01 ($^{\#\#}$), and at P<0.001 ($^{\#\#\#}$) (Compared with treated plants with 100 mM NaCl), according the One-Way ANOVA.

Transpiration rate & stomatal conductance:

Gas exchange parameters, i.e. Transpiration rate (E) and stomatal conductance (Gs) had been estimated in experimented plants in response to salinity (group two) and colonized plants by *P. indica* and salinity (group three). Moreover, these parameters appeared significant decrease in group two plants (treated by 100 mM NaCl) compared to group one plants (control). In contrast, these parameters showed a significant increases in group three plants (colonized by *P. indica* + treated by 100 mM NaCl) compared to group two plants (treated by 100 mM NaCl), in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively (Fig. 3).

Relative Water Content:

Salt treated inhibited relative water content in group two plants, while colonized plants by *P. indica* alleviate salt effects. Moreover, inhibited ratio were 7%, 6%, 3% and 3% in group two plants (treated by 100 mM NaCl) compared to group one plants (control). On other hand, alleviatory ratio were; 11%, 8%, 5% and 11%, in group three plants (colonized by *P. indica* + treated by 100 mM NaCl) compared to group two plants (treated by 100 mM NaCl), in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively (Fig. 3).

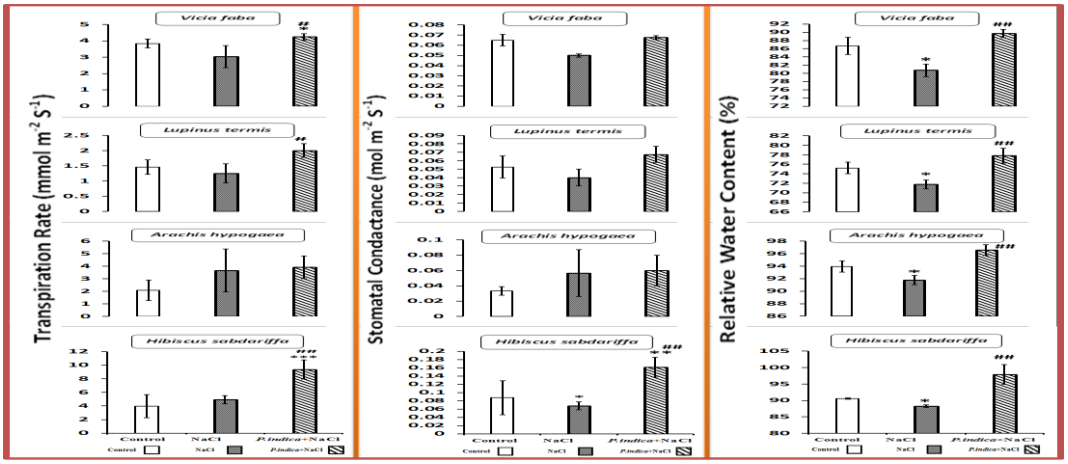

Figure 3: Effect of salinity stress (100 mM NaCl), and inoculation with *P. indica* under salinity stress (100 mM NaCl) on transpiration rate, stomatal conductance and relative water content of experimental plants. Error bar in each column equals the standard deviation from the mean. Difference is significant at P<0.05 (*), at P<0.01(**), and at P<0.001 (***) (compared with control plants). Difference is significant at P<0.05 ($^{\#}$), at P<0.01 ($^{\#\#}$), and at P<0.001 ($^{\#\#\#}$) (Compared with treated plants with 100 mM NaCl), according the One-Way ANOVA.

Protein content and profile:

Fig (4) show that change in protein fragments (soluble, insoluble and total protein) content in both group two and group three plants were non-significant in four experimental plants. But protein profile appears induced polypeptide proteins, mainly in group two plants. The results supported that an addition of *P.indica* alleviated the effects of NaCl on protein profile.

Proline content:

Proline accumulation increase in group two plants (treated by 100 mM NaCl) compared to group one plants (control). Moreover, proline accumulation decrease in group three plants (colonized by *P. indica* + treated by 100 mM NaCl) compared to group two plants (treated by 100 mM NaCl) in all plant species except in *H. sabdariffa*, where proline accumulation increase in case plants colonized by *P. indica* + treated by 100 mM NaCl compared to those treated by 100 mM NaCl (Fig. 5).

Antioxidant enzymes:

CAT and peroxidase activity were higher in group two plants shoots as compared with group one plants, and in group three plants compared with group two plants (Fig. 6).

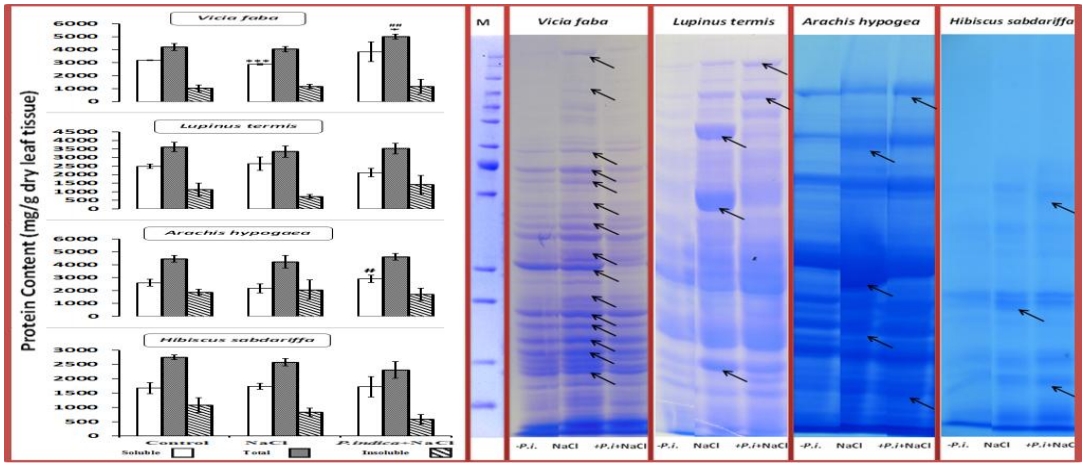

Figure 4: Effect of salinity stress (100 mM NaCl), and inoculation with *P. indica* under salinity stress (100 mM NaCl) on leaf content of protein and protein profile of experimental plants. Error bar in each column equals the standard deviation from the mean. Difference is significant at P<0.05 (*), at P<0.01(**), and at P<0.001 (***) (compared with control plants). Difference is significant at P<0.05 ($^{\#}$), at P<0.01 ($^{\#\#}$), and at P<0.001 ($^{\#\#\#}$) (Compared with treated plants with 100 mM NaCl), according the One-Way ANOVA.

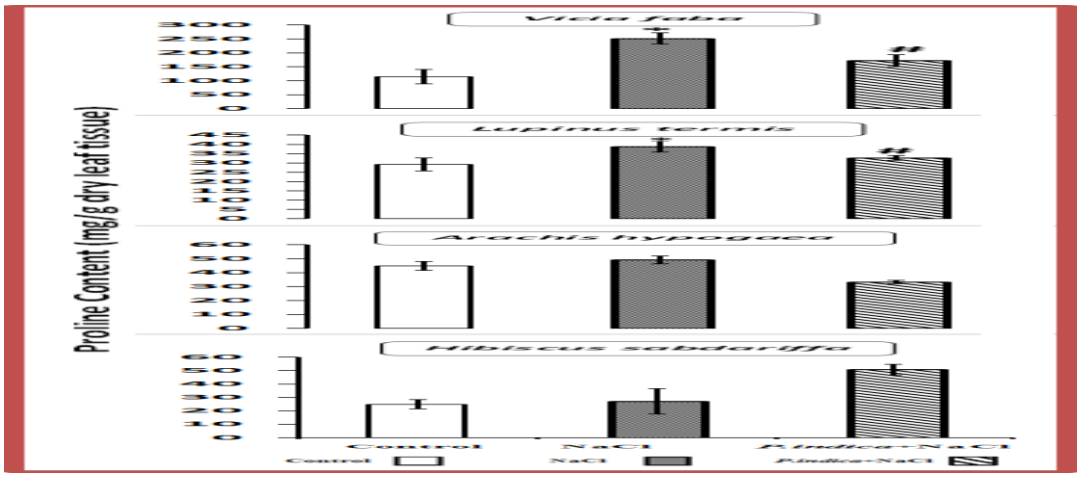

Figure 5: Effect of salinity stress (100 mM NaCl), and inoculation with *P. indica* under salinity stress (100 mM NaCl) on leaf content of proline of experimental plants. Error bar in each column equals the standard deviation from the mean. Difference is significant at P<0.05 (*), at P<0.01(**), and at P<0.001 (***) (compared with control plants). Difference is significant at P<0.05 ($^{\#}$), at P<0.01 ($^{\#\#}$), and at P<0.001 ($^{\#\#\#}$) (Compared with treated plants with 100 mM NaCl), according the One-Way ANOVA.

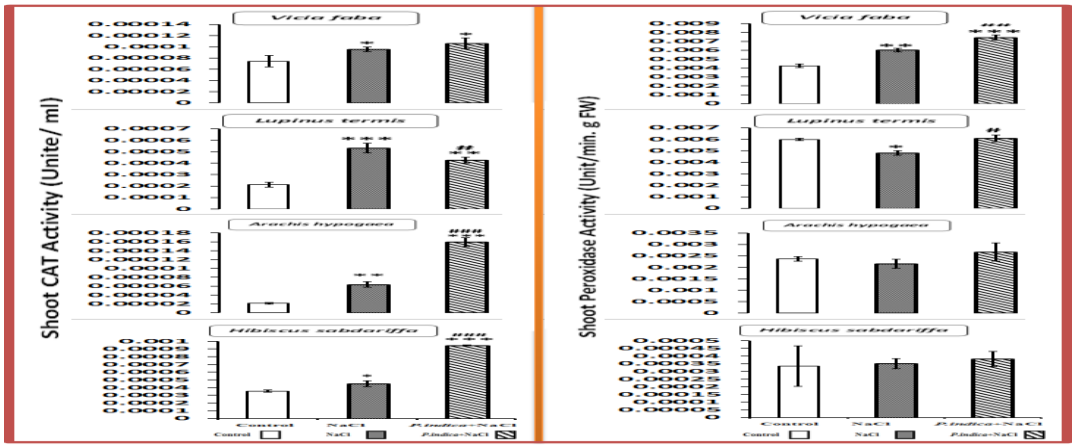

Figure 6: Effect of salinity stress (100 mM NaCl), and inoculation with *P. indica* under salinity stress (100 mM NaCl) on enzyme activity of experimental plants. Error bar in each column equals the standard deviation from the mean. Difference is significant at P<0.05 (*), at P<0.01(**), and at P<0.001 (***) (compared with control plants). Difference is significant at P<0.05 ($^{\#}$), at P<0.01 ($^{\#\#}$), and at P<0.001 ($^{\#\#\#}$) (Compared with treated plants with 100 mM NaCl), according the One-Way ANOVA.

Element content:

Effect of salinity on element (Na, Ca, K) content exhibited variable content from plant to other, and from element to other. Moreover, sodium content was increased in group two plants compared to group one plants. Increasing ratios were, 25%, 12%, 200% and 20% in *V. faba*, *L. termis*, *A. hypogaea and H. sabdariffa*, respectively. On other hand, sodium content decrease in group three plants compared to group two plants, in all experimental plant species. Decreasing ratios were, -13%, -7%, -68% and -6% in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively (Fig. 7).

Calcium under salinity stress exhibited decrease in their content in *V. faba* and *A. hypogea*, -57% and -12%, respectively. But we obtained on opposite results in other plant species, where calcium content was increase in group two compared to group one plants, in case *L. termis* and *H. sabdariffa*, 25% and 43%, respectively. In general, calcium content in group three increases than in those in group two plants. Increasing ratios were, 73%, 18%, 12% and 40%, in *V. faba*, *L. termis*, *A. hypogaea and H. sabdariffa*, respectively (Fig. 7).

Potassium under salinity stress didn't exhibit strong change in its content in group two compared to group one plants. Decreasing ratios were, -11%, -6%, -5% and -3%, in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively. Group three showed increasing in potassium content compared to group two plants. Increasing ratios were, 9%, 54%, 76% and 32%, in *V. faba*, *L. termis*, *A. hypogaea* and *H. sabdariffa*, respectively (Fig. 7).

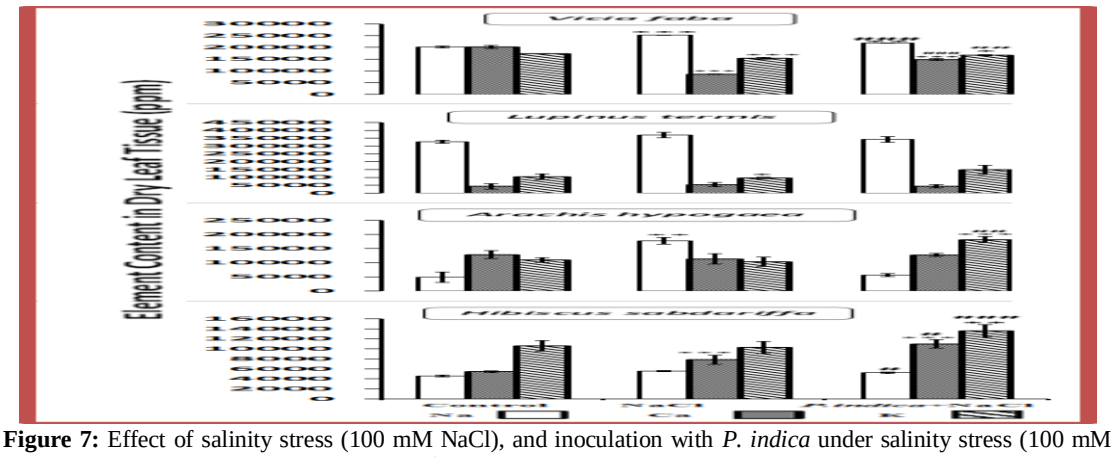

Figure 7: Effect of salinity stress (100 mM NaCl), and inoculation with *P. indica* under salinity stress (100 mM NaCl) on metal content (Na, Ca and K) of experimental plants. Error bar in each column equals the standard deviation from the mean. Difference is significant at P<0.05 (*), at P<0.01(**), and at P<0.001 (***) (compared

with control plants). Difference is significant at P<0.05 ($^{\#}$), at P<0.01 ($^{\#\#}$), and at P<0.001 ($^{\#\#\#}$) (Compared with treated plants with 100 mM NaCl), according the One-Way ANOVA.

Discussion

Growth:

It has been shown that this fungus (*P. indica*) can grow even under conditions of high salt concentration of 219.14 mM, while its growth was inhibited at salt concentration 438.27 mM (Chordia P., 2011, Kumar M., *et al*., 2012).

We analyzed the fungus' potential to protect *V. faba, L. termis, A. hypogaea* and *H. sabdariffa* from salt stress.

In the present study, the effect of salinity stress on plant biomass appeared significant decrease in experimental plant species. Moreover, this decrease in plant biomasses could alleviate by presence of *P. indica* colonized in plant roots under the same condition of salinity stress. These results agree with other observation on barley plants. Frank Waller *et al*., 2005, demonstrated that the detrimental effect of moderate salt stress on barley plants was completely abolished by *P. indica*, as shown by the fact that infested plants produced higher biomass than did non stressed control plants under these conditions. However, under high salt-stress conditions, both non infested and infested plants exhibited a severe biomass reduction.

Helmut Baltruschat *et al*., 2008, mentioned that the biomass of the youngest developed leaves of barley plants slightly decreased under saline conditions, while older leaves exhibited chlorosis and subsequent necrosis. Mild salt stress (100 mM NaCl) caused a slight, but not significant, reduction in shoot fresh weight of barley plants. However, high-salt (300 mM NaCl) treatment caused substantial biomass reduction in uncolonized and *P. indica* colonized cv. Ingrid and cv. California Mariout. Compared with uncolonized plants, shoot fresh weight of *P. indica*-colonized barley cv. Ingrid was enhanced about twofold under both control and saline conditions. Even after exposure to 300 mM NaCl, *P. indica* colonized plants produced shoot biomass comparable to uncolonized Ingrid barley grown under nonsaline conditions.

Photosynthesis process:

In this research, photosynthetic pigments content in salinity treated plants were appeared significant decreases, on other hand, *P. indica* colonized plants under the same salinity condition was showed significant increase in photosynthetic pigments.

The reduction in photosynthesis in the salinity-treated plants was reported by many researchers (Downton 1977; Ball and Farquhar 1984; Behboudian *et al*. 1986). The adverse effects of high NaCl on chlorophyll concentration have previously been shown in rice (Yeo *et al*. 1990), barley (Belkhodja *et al*. 1994), tomato (Kaya *et al*. 2001), and pepper (Kaya *et al*. 2009). In drought-induced Chinese cabbage, the presence of *P. indica* retards the decrease in the protein levels of representative components of the thylakoid membrane and of enzymes located in the plastid stroma (Sun *et al*. 2010). Sun *et al*. (2010) showed that *P. indica* retarded the drought-induced Chinese cabbage decline in the photosynthetic efficiency and the degradation of chlorophylls and thylakoid proteins. Under saline condition, *P. indica* colonization increased chlorophyll content in wheat, and the *P. indica* inoculated plants had greener leaves than non-*P. indica* inoculated plants under saline conditions (Zarea *et al*. 2011b).

Photosynthesis is important for growth and development of green plants and can be severely affected by environmental stress (Jogawat A, *et al*., 2013). In the present study, we focused on Chl and carotenoid levels, since they play a vital role in photosynthesis and photoprotection. Chl and carotenoids can be degraded due to high sodium ion toxicity during salt stress. Several crops have been reported of having reduced Chl a and Chl b concentrations upon salinity stress, e.g., cabbage (*Brassica oleracea* var. *capitata* L.), sunflower (*Heliantus annuus* L.), wheat (*Triticum aestivum* L.), and sugarcane (*Saccharum officinarum* L.)(Jamil M, *et al*.,2007; Ashraf M and Sultana R, 2000; Akram NA and Ashraf M, 2011; Arfan M, *et al*., 2007; Perveen S, *et al*., 2010; Ashraf M. and Harris PJC, 2013; Gomathi R and Rakkiyapan P. , 2011). The extent of reduction of the pigment content depends on the salt tolerance of the plant species.

In case of some plant species (wheat, pea, sunflower, etc.), the Ch content is a potential biochemical indicator of salt tolerance, although this is not true for all species and cultivars (Ashraf M. and Harris PJC, 2013). In a study with sugarcane, Gomathi and Rakkiyapan (2011) found that salt stress at various plant growth stages caused a marked reduction in Chl and carotenoid contents, but salt-tolerant varieties exhibited higher membrane stability and pigment contents.

Water relations:

Transpiration rate:

It is generally known that higher stomatal conductance in plants increases CO_2 diffusion into the leaf thereby favoring higher photosynthetic rates. Higher net CO_2 assimilation rates could in turn favour a higher biomass and higher crop yields (Taiz & Zeiger 2002; Flexas *et al*. 2002).

Pervious searches showed that transpiration rate and stomatal conductance under salt stress were decrease compared to control plants. Muhamed Ashraf *et al.*, (2003) reported that salinity cased decreased in transpiration rate and stomatal conductance in *Sesbania aculeate* and *Phaseolus vulgaris*. This supported our results, where appear that in salinity stress transpiration rate and stomatal conductance decrease compared to control plants. Moreover, colonized plants by *P. indica* under salinity condition could overcome on transpiration rate and stomatal conductance decreases.

Relative water content:

In the present study, leaf relative water content in *V. faba, L. termis, A. hypogaea* and *H. sabdariffa* plants under salinity stress was less than in control plants. Moreover, relative water content in colonized plants under saline condition was higher than plants under salinity stress. Kumar *et al*. (2009) also reported that relative water content in the leaves of wheat was significantly higher in *P. indica* inoculated than in non-inoculated wheat plants under saline conditions.

Proline:

Under salt stress, plants accumulate some organic solutes such as proline and inorganic ions to maintain higher osmotic adjustment (Yang *et al*. 2009). Free amino acids are important osmolytes contributing to osmotic adjustment in plants (Hajlaoui *et al*. 2010). With increasing external salt concentration, free amino acids accumulate in the leaves and roots of maize (Abd-El Baki *et al*. 2000; Neto *et al*. 2009; Hajlaoui *et al*. 2010). Among free amino acids, proline is a contributor to osmotic adjustment in salt-stressed maize plants (Hajlaoui *et al*. 2010). It appears that the presence of the *P. indica* fungus in the roots may modify the osmotic potential of the leaves as they have been shown to influence the composition of the level of proline (Zarea *et al*. 2011b). Proline accumulation is thought an adaptive feature under salinity stress in *P. indica* (Zarea *et al*. 2011b). Results also show that the accumulation of proline in wheat is increased by *P. indica* inoculation (Zarea *et al*. 2011b). The high level of proline enables the plants to maintain osmotic balance when growing under low water potentials (Stewart and Lee 1974). Proline acts as a major reservoir of energy and nitrogen for utilization by plants subjected to salinity stress (Goas *et al*. 1982; Ashraf and Foolad 2006). In this search, proline accumulations in stressed plants by salinity were higher than control plants. *Vicia faba*, *Lupinus termis* and *Arachis hypogaea* colonized plants by *P. indica* under salinity conditions were proline accumulations less than in non-colonized plants. But *Hibiscus sabdariffa* colonized plants by *P. indica* under salinity conditions were proline accumulations more than in non-colonized plants.

Enzyme activity:

Damage to plants that are induced by salt stress may also be a consequence of the production of ROS (reactive oxygen species) (Hernandez *et al*. 1995). In this regard, plants with high concentrations of antioxidants or antioxidative enzymes are typically more resistant to damage by ROS (Spychalla and Desbough 1990; Dionisio- Sese and Tobita 1998; Jiang and Zhang 2002). Plants are endowed with an array of radical scavengers and antioxidant enzymes that act in concert to alleviate oxidative stress. The induction of ROS-scavenging enzymes, such as superoxide dismutase (SOD), peroxidases (POXs), and catalase (CAT), is the most common mechanism for detoxifying ROS synthesized during stress responses (Wojtaszek 1997; Mittler 2002). An imbalance between antioxidant defenses and the amount of ROS results in cellular injury (Foyer and Noctor 2000). An increasing

body of evidence suggests that high salinity induces oxidative stress in plants that is at least partly responsible for tissue damage (Herna´ndez *et al*. 2000; Mittova *et al*. 2004). Several studies have demonstrated that salinity increases antioxidant activities in salt-tolerant plants above the levels found in salt-sensitive plants (Gossett *et al*. 1994; Gueta-Dahan *et al*. 1997; Mittova *et al*. 2004). Symbiotic *P. indica* fungus may promote the activation by plants antioxidant enzymes to scavenge the ROS (reactive oxygen species) (Rodriguez and Redman 2005; Baltruschat *et al*. 2008; Kumar *et al*. 2009). Increased antioxidant enzyme activity, including CAT (catalase), APX (ascorbate peroxidase), DHAR (dehydroascorbate reductase), MDHAR (monodehydroascorbate reductase), and GR (glutathione reductase), plays a significant role in tolerance to abiotic stressors. Baltruschat *et al*. (2008) point out that these enzyme activities are maintained at a high level in *P. indica* infected plants but decrease gradually in uninfected plants. *P. indica* stimulates antioxidant enzyme activities and prevents ROS formation by retarding the degradation of polyunsaturated lipids (Sun *et al*. 2010). However, compared to APX, CAT, and DHAR antioxidant enzymes, GR activity was the least affected by *P. indica* (Baltruschat *et al*. 2008; Kumar *et al*. 2009). Several reports have demonstrated that antioxidant enzyme activities are crucial for *P. indica* induced resistance against abiotic stress (Baltruschat *et al*. 2008, Vadassery *et al*. 2009; Sun *et al*. 2010).

Previous results were supported our results. Where, under salinity conditions antioxidant enzyme activity (CAT and POX) increased compared to control plants. Moreover, colonized plants by *P. indica* under salinity conditions shown increasing in antioxidant enzyme activity compared to non-colonized plants under salt stress, in all experimental plants species.

Element content:

It has been generally accepted that Am fungi would enhance nutrient uptake by infected plants under salinity conditions (Roa and Tak, 2002; Yano-Melo *et al*., 2003; Zandavalli *et al*., 2004).

Plants can use three strategies for the maintenance of low cellular/tissue Na^+ concentration: sodium exclusion, sodium compartmentalization, and sodium secretion (Zhang *et al*. 2001). Sodium transport out of the cell can take place by the operation of plasma membrane-bound Na^+/H^+ antiports. Transport mechanisms can also actively move ions across the tonoplast into the vacuole, removing the potentially harmful ions from the cytosol. These ions, in turn, act as an osmoticum within the vacuole (Shi *et al*. 2000; Mansoor *et al*. 2003). In this search, sodium content was increased in group two plants (treated by 100 mM NaCl) compared to group one plants (control), in all experimental plant species. But, sodium content decrease in colonized plants by *P. indica* compared to non-colonized plants, under salinity conditions in all experimental plant species.

Rabie *et al*., (2005) reported that AM (arbuscular mycorrhiza) faba plants contained significant higher levels of K^+, Mg^{2+} and Ca^{2+} ions, particularly in the presence of NFB, than non-Am plants at all salinity levels. Based on their results and on existing literatures, the greater salt tolerance of Am plants may be the result of the plant nutrition improvement under salinity stress. It is also noteworthy that Na^+ concentration in shoot system of AM *faba* plants, especially in the presence of NFB, at high salinity level was comparable to that of non-Am plants at moderate salinity level. These results are consistent with the previous work (Zandavalli *et al*., 2004; Rabie *et al*., 2005), and suggest that Am fungi may protect shoot system, mainly leaves, from Na^+ toxicity either by regulating Na^+ uptake from the soil or by accumulating it in root thereby delaying its translocation onto shoot system of infected plants. This may reflect the potential role of Am fungi for increasing plant K^+ and Ca^{2+} uptake more than Na^+ under salinity stress. The role of K^+ and Ca^{2+} in salt adaptation of plants has been previously discussed by several authors. Parida and Das (2005) reported that when under salt stress; plants maintain high concentrations of K^+ and low concentrations of Na^+ in the cytosol. They do this by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force for transport. In addition, externally supplied Ca^{2+} reduces the toxic effects of NaCl, presumably by facilitating higher K^+/Na^+ selectivity.

High salinity also results in increased cytosolic Ca^{2+} that is transported from the apoplast and intracellular compartments. The resultant transient Ca^{2+} increase potentialities stress signal transduction and leads to salt adaptation. Based on these data and existing literatures it is conceivable to conclude that Am symbioses may regulate the expression and activate K^+ and Na^+ transporters and H^+ pumps that generate the driving force for transport. Besides, it may increase the transient Ca Ca^{2+} from apoplast and intracellular compartments. This inference needs further investigation to support it. Rabie *et al*., (2005) reported that AM *faba* plants grew in high saline environments without any detectable signs of toxic effects of NaCl, reflecting adaptation to high salinity. These extrapolations emphasized the prime role of Am fungi in increasing salinity tolerance of *faba* plants up to 6.0 dSm^{-1}. These data suggest that selective accumulation or exclusion of ions, control of ion uptake by roots and transport into leaves and compartmentalization of ions at the cellular and whole plant levels are the most effective strategies of AM fungi for adaptation of *faba* plants to salinity stress.

In the present search, calcium under salinity stress exhibited decrease in their content in *V. faba* and *A. hypogea*. But we obtained on opposite results in other plant species, where calcium content was increase in group two(treated with 100 mM NaCl) compared to group one plants (control), in case *L. termis* and *H. sabdariffa*. In general, calcium content in group three (*P. indica* + 100 mM NaCl) was increase than in those in group two plants (treated with 100 mM NaCl). Potassium under salinity stress didn't exhibit strong change in its content in group two compared to group one plants. Group three showed increasing in potassium content compared to group two plants.

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الملخص العربى:

قد تبين أن المعاملة بفطر البرفورموسبورا انديكا لبعض نباتات المحاصيل مثل الفول البلدى، و الترمس، والفول السودانى، و الكركدية تظهر مقاومة للاجهاد الملحى بالنسبة للنباتات الغير معاملة بالفطر. حيث أن وجود الفطر يقلل من تأثير الأجهاد الملحى على معدل النمو، و يحسن عملية البناء الضوئى ويزود من المحتوى البروتينى لتلك النباتات. علاوة على ذلك، يحسن العلاقات المائية للنباتات. و من ذلك نجد أن الفطر يكون له تأثير جيد على تخفيف الآثار السيئة للملوحة على النباتات. وبالتالى من النتائج السابقة يمكننا القول بأنه يمكن لنباتات الفول البلدى، و الترمس، والفول السودانى، و الكركدية أن تزرع فى أراضى متوسطة الملوحة و ذلك فى وجود فطر البرفورموسبورا انديكا.